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Featured Article

Effects of *APOE*-ε4 allele load on brain morphology in a cohort of middle-aged healthy individuals with enriched genetic risk for Alzheimer's disease

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Abstract

Introduction: Apolipoprotein E (*APOE*)-ε4 is the major genetic risk factor for Alzheimer's disease. However, the dose-dependent impact of this allele on brain morphology of healthy individuals remains unclear.

Methods: We analyzed gray matter volumes (GMvs) in a sample of 533 healthy middle-aged individuals with a substantial representation of ε4-carriers (207 heterozygotes and 65 homozygotes).

Results: We found *APOE*-ε4 additive GMv reductions in the right hippocampus, caudate, precentral gyrus, and cerebellar crus. In these regions, the *APOE* genotype interacted with age, with homozygotes displaying lower GMv after the fifth decade of life. *APOE*-ε4 was also associated to greater GMv in the right thalamus, left occipital gyrus, and right frontal cortex.

Discussion: Our data indicate that *APOE*-ε4 exerts additive effects on GMv in regions relevant for Alzheimer's disease pathophysiology already in healthy individuals. These findings elucidate the mechanisms underlying the increased Alzheimer's disease risk in ε4-carriers, suggesting a dose-dependent disease vulnerability on the brain structure level.

Keywords: Alzheimer's disease; *APOE*-ε4; Healthy individuals; Voxel-based morphometry; Gray matter volumes; Aging

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1. Background

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder characterized by progressive cognitive impairment and a characteristic pattern of regional brain atrophy that starts in the hippocampus and medial temporal regions and then spreads to other cortical areas [1]. The two major neuropathologic features of AD are extracellular fibrillary amyloid β (Aβ) plaques and intracellular neurofibrillary tau tangles. AD pathology develops slowly with a protracted preclinical phase characterized by abnormal cerebral Aβ deposition in cognitively intact individuals [2]. The apolipoprotein E (*APOE*)-ε4 represents the major genetic risk factor for late-onset AD, with increasing copies of the ε4 allele being associated to greater AD risk and younger age of disease onset [3,4]. Three common polymorphic alleles referred as ε2, ε3, and ε4 code, respectively, three distinct apoeE isoforms, which differ among each other by a single amino acid, and depending on the allele pairs, they result in six possible diploid haplotypes. In the brain, apoE mediates neuronal delivery of cholesterol, which is an essential component for axonal growth, synaptic formation, and remodeling, and has an important role in Aβ metabolism. The apoE4 isoform (coded by the *APOE*-ε4 allele) has been shown to be less efficient in Aβ clearance and cholesterol transport than the other isoforms (apoE2 > apoE3 > apoE4) [4]. *APOE*-ε4-homozygous (HO) individuals are therefore an interesting population for studying the long-term effects of the lack of expression of the more efficient apoE isoform. Regarding the pathogenesis of AD, the mechanisms through which *APOE*-ε4 increases risk can be regarded as conferring a loss of neuroprotective function, gain of neurotoxic function, or both [4]. *APOE*-ε4 allele dose effects on memory decline have been reported in cognitively healthy individuals [5], and a dose-dependent hippocampal gray matter (GM) degeneration has been observed in AD patients [6,7]. However, only a few reports have assessed the impact of *APOE*-ε4 homozygosity on the brain morphology of healthy middle-aged subjects. One cross-sectional study found significantly reduced hippocampal volume in healthy *APOE*-ε4 homozygotes, as compared to ε4-heterozygotes and noncarriers (NC), without however reporting any ε4 dose-dependent effects [8]. In the follow-up of this study, an age-related hippocampal volume loss was found only in homozygotes, again not supporting for *APOE*-ε4 additive effects in this region [9]. On the other hand, Chen et al. [10] reported a significant correlation between *APOE*-ε4 gene dose and higher annualized rates of whole-brain atrophy in healthy individuals.

Aside from these reports, most of the studies in the literature pooled together *APOE*-ε4 homozygotes and heterozygotes into a single *APOE*-ε4 carrier category. Although longitudinal studies reported significantly faster decays of regional gray matter volume (GMv) in healthy carriers compared to NC [11,12], most cross-sectional studies which determined volumes in *a priori*–defined AD-sensitive cerebral regions did not find significant group differences [13–15]. Similarly, null findings were reported in studies using voxelwise techniques, such as voxel-based morphometry (VBM) [16–18]. By contrast, two studies using VBM reported GMv differences in brain regions including the hippocampus, lingual gyrus, and precuneus, when comparing carriers to NC [19,20]. More recently, Ten Kate et al. [21] reported decreased volume in the precuneus and insula in healthy middle-aged carriers of the risk allele as well as an interaction between *APOE* status and age in determining GMv in temporal and occipital regions. Discrepancies among studies might be in part due to the volumetric technique adopted and the difference in the samples' age, where studies including relatively older individuals tend to report significant differences compared to those including younger samples.

In the present study, we sought to determine the effects of *APOE*-ε4 allele load on brain morphology to better characterize the mechanisms through which *APOE*-ε4 confers an increased risk to develop AD in the healthy population. To this end, we used VBM in a cohort of healthy middle-aged individuals enriched for this genetic risk for AD (261 NC, 207 *APOE*-ε4 heterozygotes, and 65 *APOE*-ε4 homozygotes). We also sought to determine potential interactions between *APOE* status and age in determining GMv variability.

2. Methods

2.1. Study participants

The recruitment for the study consisted of two steps. First, 2743 cognitively healthy volunteers aged between 45 and 76 years were enrolled in the ALFA (ALzheimer and FAmilies) study, a large cohort program pointing to the identification of neuroimaging biomarkers of preclinical AD in the general population [22]. Exclusion criteria included performance exceeding established cutoff for a number of cognitive tests and presence of a psychiatric diagnosis [22]. Second, after *APOE* genotyping, all participants homozygous for the ε4 allele as well as carriers of the ε2 allele were invited to undergo magnetic resonance imaging (MRI) scanning along with ε4-heterozygous (HE) and NC matched for age and sex. This sampling strategy resulted in 576 study participants, of which 43 had to be discarded due to either MRI incidental findings or poor image quality, resulting in the final sample included in our study of 533 individuals. Demographic characteristics of the participants are summarized in Table 1 and Supplementary Table 1. For the statistical analyses, participants were pooled according to the cumulative presence of the ε4 allele, that is, NC, HE, and HO. Total intracranial volume as well as gender and education years did not differ among the three groups. However, HO individuals were significantly younger than NC and HE

Table 1
Sample characteristics

	Total sample (N = 533)		NC (N = 261)		HE (N = 207)		HO (N = 65)		
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	Inferential statistics
Age*	57.58	7.43	57.93	7.53	58.22	7.41	54.14	6.18	$F = 8.22$; $P < .01$
Education*	13.64	3.56	13.69	3.62	13.66	3.53	13.38	3.46	$F = 0.19$; $P = .82$
TIV (cm^3)	1490.11	146.07	1484.96	155.17	1495.41	137.73	1493.91	135.20	$F = 0.32$; $P = .72$
Male/female	213/320		95/166		94/113		24/41		$\chi^2 = 4.19$; $P = .13$

Abbreviations: NC, noncarriers; HE, ε4-heterozygous; HO, ε4-homozygous; M, mean; SD, standard deviation; TIV, total intracranial volume.
*Indicated in years.

(Table 1). For this reason, age was included as covariate in all subsequent analyses. The study was approved by the local ethics committee, and all participants gave written informed consent to participate in the study.

2.2. APOE *genotyping*

Total DNA was obtained from the blood cellular fraction by proteinase K digestion followed by alcohol precipitation. Samples were genotyped for two single-nucleotide polymorphisms, *rs429358* and *rs7412*, determining the possible *APOE* isoforms: ε1, *rs429358* (C) + *rs7412* (T); ε2, *rs429358* (T) + *rs7412* (T); ε3, *rs429358* (T) + *rs7412* (C); and ε4, *rs429358* (C) + *rs7412* (C). Of the 533 participants, 163 were ε3/ε4 carriers, 149 were homozygous for the ε3 allele, 105 were ε2/ε3 carriers, 65 were homozygous for the ε4 allele, 44 were ε2/ε4, and 7 were ε2/ε2 carriers. The allele frequencies were in Hardy-Weinberg equilibrium.

2.3. *Image data acquisition and preprocessing*

MRI was conducted with a 3T General Electric scanner (GE Discovery MR750 W). Structural 3D high-resolution T1-weighted images were collected using a fast spoiled gradient-echo sequence implementing the following parameters: voxel size = 1 mm^3 isotropic, repetition time = 6.16 ms, echo time = 2.33 ms, inversion time = 450 ms, matrix size = 256 × 256 × 174, and flip angle = 12°. Images were segmented into GM tissue using the new segment function implemented in Statistical Parametric Mapping software (SPM12; Wellcome Department of Imaging Neuroscience, London, UK) and located into a common space for subsequent normalization using a 9-affine parameter transformation. GM images were then used to generate a reference template object of the sample, which was warped into a standard Montreal Neurological Institute space using the high-dimensional DARTEL toolbox [23]. The generated flow fields and normalization parameters were then implemented to normalize the native GM T1 images to the Montreal Neurological Institute space. Jacobian determinants were applied to preserve the local native amount of GM (modulated images). Quality control of normalization was assured by checking the sample homogeneity with the computational anatomy toolbox (CAT12) (http://dbm.neuro.uni-jena.de/cat/) using non-smoothed data, which did not return errors in the registration procedure in any subject. Finally, images were spatially smoothed with a 6-mm full width at half maximum gaussian kernel. Total intracranial volume was computed by summing the segmented GM, white matter, and cerebrospinal fluid for each individual.

2.4. *Neuropsychological assessment*

Global cognition was assessed using the Mini–Mental State Examination (MMSE) [24], whereas episodic memory was assessed with the Memory Binding Test [25], and executive functions were evaluated using the Wechsler Adult Intelligence Scale [26]. We computed a global score of immediate recall by summing up all individual scores from the total paired recall and total free recall scales of the Memory Binding Test. Similarly, a global index of delayed recall was obtained by summing scores of total delayed paired recall and total delayed free recall.

2.5. *Statistical analyses*

The normalized, modulated, and smoothed GM images were entered in a multiple regression design in SPM12. We partitioned genetic variance by including in the design matrix two independent dummy regressors coding both additive and other genotypic effects of *APOE*-ε4, as proposed for analyzing quantitative trait loci [27,28] and as previously implemented in neuroimaging studies [7]. Briefly, a genetic additive model predicts an incremental response of the quantitative trait according to the allelic load, whereas a dominant model predicts a common response to 1 or 2 copies of the risk allele. Finally, a recessive model predicts a common response to 0 or 1 copy of the risk allele. Linear and quadratic expansions of age, as well as sex, years of education, and total intracranial volume, were included as covariates. To control for multicollinearity, age and age squared were centered at their respective mean. Because previous studies reported significant interactions between *APOE* status and age in determining GMv variability [21], we fitted the model

Table 2
Significant GMv differences between *APOE*-ε4 carriers and noncarriers for the hypothesis-driven and whole-brain analyses

Brain region	*t*-value*	*K*†	$P_{uncorrected}$	P_{FWE}	MNI coordinates		
					x	y	z
Hypothesis-driven analysis							
Genotypic model							
Right hippocampus	−5.11	137	<.001	.003	21	−36	8
Right thalamus	4.67	192	<.001	.02	12	−12	6
Additive model							
Right hippocampus	−4.91	154	<.001	.008	21	−36	8
Right thalamus	4.47	214	<.001	.04	12	−12	6
Whole-brain analysis							
Genotypic model							
Right hippocampus	−5.11	194	<.001	.01	21	−36	8
Right cerebellar crus	−4.14	102	<.001	.37	53	−53	−44
Right caudate	−3.70	157	<.001	.88	11	14	11
Right thalamus	4.67	193	<.001	.06	12	−12	6
Left middle occipital	4.33	248	<.001	.21	−19	−96	9
Right superior frontal	3.75	122	<.001	.84	23	48	21
Additive model							
Right hippocampus	−4.91	209	<.001	.02	21	−36	8
Right cerebellar crus	−3.93	101	<.001	.64	51	−53	−44
Right precentral	−3.85	117	<.001	.73	45	−6	59
Right caudate	−3.69	102	<.001	.88	11	20	8
Right thalamus	4.42	202	<.001	.16	12	−12	6
Left middle occipital	4.19	208	<.001	.33	−19	−96	9

Abbreviations: APOE, apolipoprotein E; GMv, gray matter volume; MNI, Montreal Neurological Institute; P_{FWE}, *P* value corrected for multiple comparisons using a family-wise error rate approach.

*Negative *t*-values indicate *APOE*-ε4-related GMv reductions, whereas negative values indicate opposite effects.

†Cluster size indicated in number of contiguous voxels.

with the interaction terms *APOE* × age and *APOE* × age squared. Additive and genotypic effects of *APOE*-ε4 on GM volumes were separately assessed with *t*-test contrasts on the respective regressors. Potential interactions between *APOE* status and age were assessed with *t*-tests implemented on both linear and quadratic interaction terms in the design matrix. To disentangle the specific contribution of each *APOE* haplotype in determining GMv group differences, we performed a separate model where five orthogonal dummy regressors were entered as independent predictors. Because the number of individuals homozygous for the ε2 allele was only seven, we collapsed this group with the ε2/ε3 genotype group yielding a single category that we coded as ε2/ε2 + ε2/ε3.

First, we performed a hypothesis-driven analysis that was restricted to all voxels within an *a priori*–defined anatomical region of interest (ROI) that included cerebral areas specifically affected by AD, as recently reviewed by Chapleau et al. from previous VBM studies [1]. This mask included the following bilateral regions: hippocampus, thalamus, precuneus, posterior cingulum, angular gyrus, inferior and middle temporal gyri, superior temporal pole, middle occipital cortex, insula, fusiform gyrus, and the straight rectus. For this analysis, we performed a small volume correction as implemented in SPM12, selecting a primary voxelwise threshold of uncorrected $P < .001$ and successively applying a correction for multiple testing using a family-wise error rate (FWE) approach [29]. Second, we performed an unbiased whole-brain analysis to detect additional effects of *APOE*-ε4 in our genetically enriched sample. For this exploratory analysis, results were considered significant if surviving a voxelwise statistical threshold of uncorrected $P < .001$, with a cluster-extent threshold of 100 voxels. This cutoff is reliably conservative and further protects against type I error [30,31]. The effect size of the *APOE*-ε4 load on GMv was computed voxelwise by converting *F*-maps into equivalent correlational effects (*r*-values). All cognitive measures were entered in a univariate analysis of variance with the independent factor being the number of ε4 alleles (3 levels) and additionally controlling for age, sex, and years of education. For visualization of the statistical parametric maps, we used xjView (http://www.alivelearn.net/xjview).

3. Results

3.1. *Main effect of* APOE *genotype*

When assessing genotypic effects, the ROI analysis returned significant results in the right posterior hippocampus and right medial thalamus. Specifically, compared to NC, ε4-carriers demonstrated a lower GMv in the right posterior hippocampus while showing greater

GMv in the right medial thalamus. Both of these regions were also significant when assessing ε4 allele additive effects, indicating that ε4-carriers showed reduced posterior right hippocampal and increased right thalamic volume in a dose-dependent manner (Table 2).

The exploratory whole-brain analysis confirmed the significantly additive reduced GMv in carriers compared to NC in the right posterior hippocampus and additionally revealed significant ε4 additive effects in the same direction in the right caudate nucleus, right precentral gyrus, and right cerebellar crus (Table2; Fig. 1A, 1B, and 1D). The right hippocampus survived FWE correction also in this unbiased analysis (P_{FWE} = 0.023). In addition, the whole-brain analysis confirmed the dose-dependent hypertrophy in carriers compared to NC in the right medial thalamus and also yielded an additive effect of *APOE*-ε4 in the left middle occipital gyrus as well as a genotypic effect in the same direction for the right superior frontal cortex (Table 2; Fig. 1A, 1C, and 1E). Removing the ε2/ε4 carriers from the analysis did not significantly alter the results. Supplementary Fig. 1 shows the effects size of the additive and genotypic effects of *APOE*-ε4 allele load across the whole brain, which were relatively small to moderate and displayed a symmetric pattern. Supplementary Table 2 summarizes GMv difference among the five *APOE* genotype groups, as revealed by planned *t*-test comparisons. Presence of the *APOE*-ε4 was not significantly associated to cognitive performance in any of the analyzed cognitive domain (Supplementary Table 3). Furthermore, we observed no significant difference in cognitive performance among each of the five *APOE* genotype groups (Supplementary Table 4).

To better investigate whether *APOE*-ε4-allelic load modulates the association between GMv and cognitive performance, we performed a post hoc analysis by modeling the interaction between *APOE* status and scores of episodic memory as well as executive function. In so doing, we performed an ROI analysis in SPM using an anatomical mask, which included the clusters where we observed the main effects of *APOE*-ε4 described previously. Given the exploratory nature of this post hoc ROI analysis, we selected a voxelwise primary threshold of $P < .05$. We observed a significant interaction between *APOE* status and executive function performance, specifically the HO individuals were the only group displaying a negative association between GMv in the caudate as well as the cerebellar crus and scores in two subscales of the Wechsler Adult Intelligence Scale (Digit-Symbol Substitution Test and Digit-Span Sequencing; Supplementary Fig. 2). No significant interactions were observed in the selected ROIs between *APOE* status and episodic memory.

3.2. *Interaction between* APOE *genotype and age*

When assessing genotypic effects, the hypothesis-driven analysis returned a significant interaction between *APOE* genotype and age in the right posterior hippocampus ($t_{521} = -4.82$, cluster size [*k*] = 234, P_{FWE} = .01). The whole-brain analysis assessing genotypic effects confirmed the significant interaction in the right posterior hippocampus, which also survived FWE correction (P_{FWE} = .034), and additionally revealed an interaction in the right caudate ($t_{521} = -3.80$, $k = 198$, $P < .001$), as well as the right ($t_{521} = -5.53$, $k = 268$, $P < .001$) and left ($t_{521} = -4.06$, $k = 157$, $P < .001$) cerebellar crus (Fig. 2A and 2B). The interaction effects in all these four regions were also significant when assessing ε4 additive effects ($P < .001$, $k > 100$), indicating an *APOE*-ε4 dose-related regional GMv reduction over age in our cross-sectional design. The effect size for the interaction between *APOE* status and age was small to moderate and showed symmetric pattern resembling that of the main effects (Supplementary Fig. 1).

4. Discussion

We have reported that the *APOE*-ε4 genotype significantly impacts on GMv in a group of 533 healthy middle-aged individuals. We capitalized on a cohort harboring a significantly higher number of *APOE*-ε4 homozygotes than previously reported in single-site studies of healthy subjects. This allowed us to reliably assess the effects of the risk allele on gray matter morphology. We observed a highly symmetrical morphological pattern, of a small-to-moderate effect size, associated to the additive effects of *APOE*-ε4 load across the whole brain, although these effects were mainly driven by the homozygous group (Figs. 1 and S1).

We found a main effect of *APOE*-ε4 in determining GMv reduction of the posterior hippocampus, in a dose-dependent fashion. In this region, we also observed a significant interaction with age, where the HO group displayed a departure of hippocampal GMv after the fifth decade of life, with respect to both NC and HE. Our interaction findings resemble previous data reported by Caselli et al. [5] who found a significant linear relationship between the ε4-allelic load and the age-related memory decline in cognitively intact individuals and may in fact represent a brain structural correlate of those results. However, we did not find a significant impact of the *APOE*-ε4 risk variant on cognitive performance. Such a null finding matches previous works documenting moderate if not null effects of the ε4 allele on cognitive performance in healthy middle-aged individuals when examined cross-sectionally [32,33]. Hippocampal degeneration is the most typical characteristic of AD, where hippocampal atrophy rates positively correlate with the *APOE*-ε4 allele load [34]. In addition, a lower hippocampal volume in nondemented individuals has been reported to increase the risk of developing AD [35,36] and to be associated to higher cerebrospinal fluid tau levels [37]. In particular, the posterior hippocampus is among the first regions suffering structural

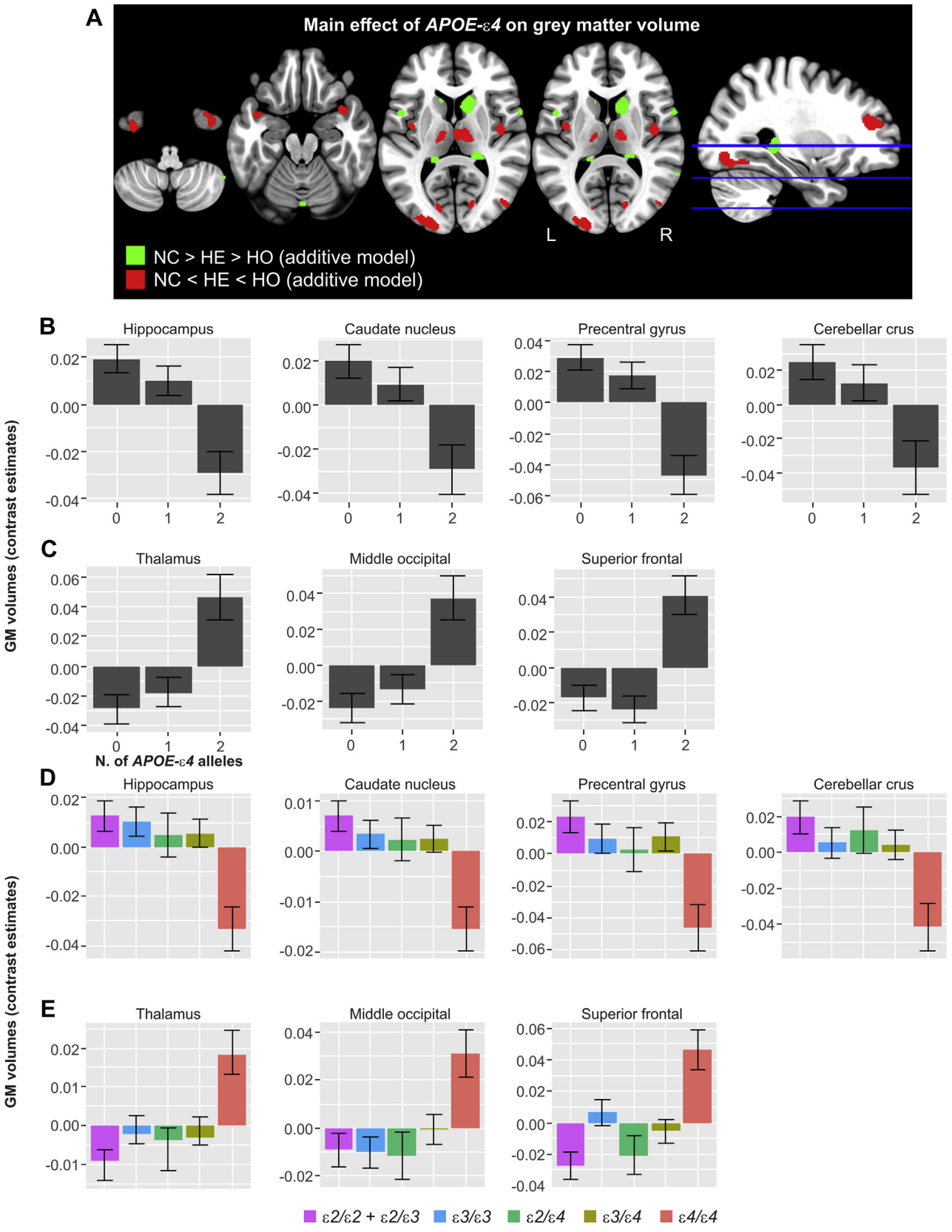

Fig. 1. *APOE*-ε4 genotype impacts on GMv in healthy middle-aged individuals. (A) Statistical parametric maps showing the brain regions where the *APOE*-ε4 allele had a significant impact on GMv. For visualization purposes, the maps are thresholded at $P < .005$ uncorrected for multiple testing. Green-colored areas indicate additive effects of the risk allele in determining GMv reductions, whereas red-colored areas indicate opposite effects. In the whole brain, we observed a

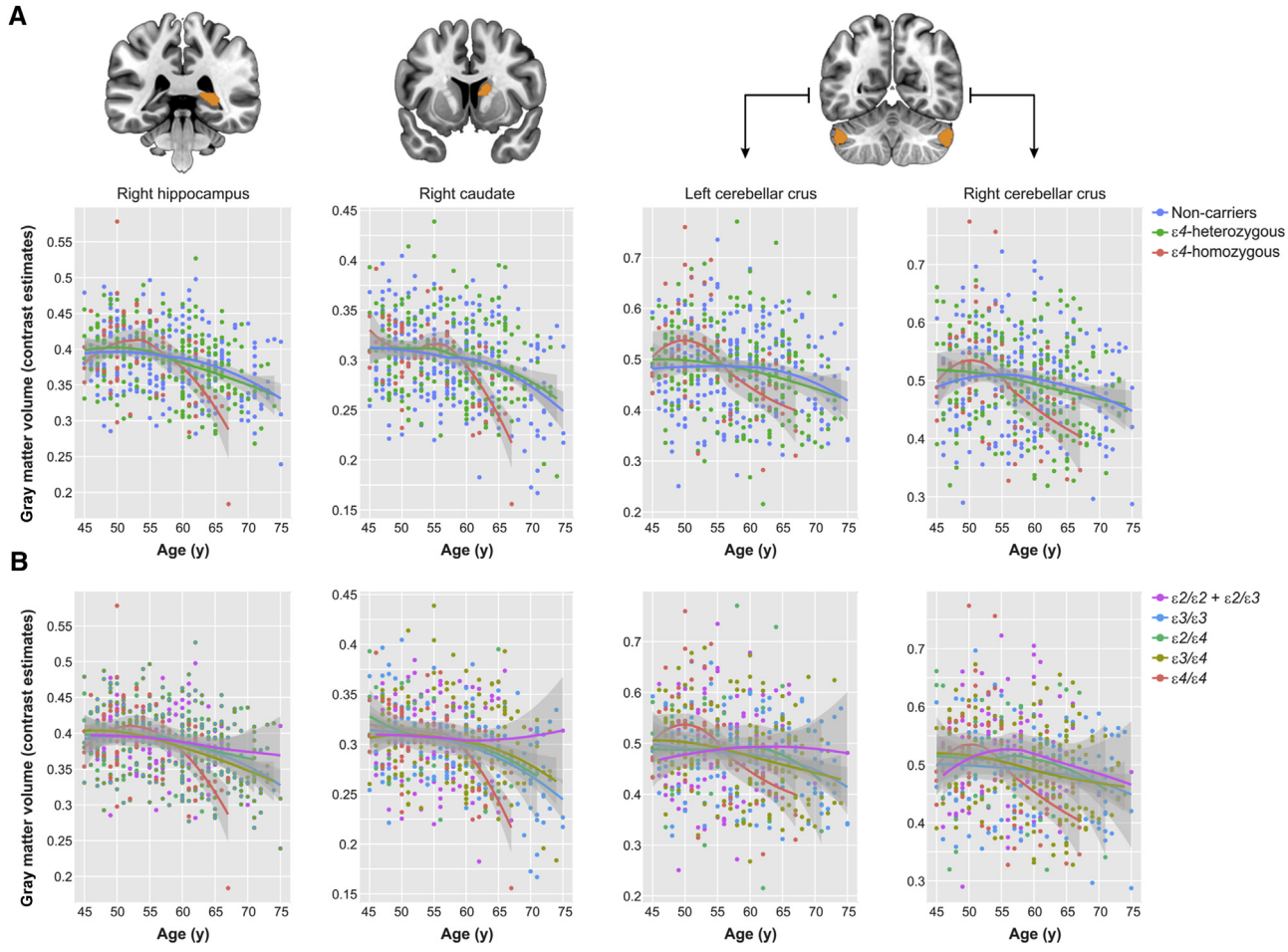

Fig. 2. Significant interactions between *APOE* genotype and age in determining regional GMv variability in healthy individuals. (A) *APOE*-ε4 carriership significantly interacted with age in four brain regions, determining distinct age-related trajectories of GMv depending on the number of the risk alleles. Significant interactions were found in the right hippocampus, right caudate nucleus, and in the right and left cerebellar crus. In these regions, *APOE*-ε4 was related to steeper nonlinear decline of GMv across age, with homozygous showing the greatest effect. The age-related GMv trajectories diverged among groups between the fifth and sixth decades of life. For visualization purposes, nonparametric smoothing spline functions were used to fit the data. Shaded gray areas indicate 90% confidence intervals. (B) Same as in (A), with the *APOE* genotype groups broken down into five categories. Abbreviations: APOE, apolipoprotein E; GMv, gray matter volume.

degeneration in the earliest phases of AD and specifically undergoes GMv loss in AD but not in other types of dementias [1]. In fact, HO individuals present a significantly younger mean age of onset of AD (68 years) [3,4]. Together with our findings, this would suggest that the older *APOE*-ε4 homozygotes in our sample might have already started the neurodegenerative process, although with cognition still within normal ranges. However, other effects of the *APOE*-ε4 allele that may precede or be independent of amyloid deposition have been described in the literature, such as impaired cholesterol delivery to the brain to sustain synaptic integrity and plasticity, promotion of proinflammatory responses or to maintain vascular health [4]. Therefore, an alternative explanation to our finding would be that the ε4 allele load renders the brain tissue more vulnerable to insults inherently provoked by aging, but not necessarily associated to AD pathology. Longitudinal multimodal studies with the acquisition of

highly symmetrical pattern of effects exerted by the *APOE*-ε4 genotype. (B) Mean-centered bar plots of the adjusted GMv in the regions where the effect of the risk allele survived statistical threshold, determining lower *APOE*-ε4 dose-dependent GMv. Error bars indicate SEM. (C) Mean-centered bar plots of the adjusted GMv in the regions where the effect of the risk allele survived statistical threshold, determining higher *APOE*-ε4 dose-dependent GMv, except for the superior frontal cortex where the effects were recessive with the ε4 allele. Error bars indicate SEM. (D) Mean-centered bar plots of the adjusted GMv in the regions reported in (B), across five different *APOE* haplotypes. Error bars indicate SEM. (E) Mean-centered bar plots of the adjusted GMv in the regions reported in (C), across five different *APOE* haplotypes. Error bars indicate SEM. Abbreviations: GMv, gray matter volume; NC, noncarriers; HE, ε4-heterozygous; HO, ε4-homozygous; SEM, standard error of the mean.

core AD biomarkers are therefore needed to elucidate the contribution of *APOE*-ε4 to these distinct biological mechanisms.

Previous cross-sectional structural MRI studies conducted in healthy individuals found discordant results on the relationship between *APOE*-ε4 and hippocampal volume [38]. Former cross-sectional [8] and longitudinal studies [9] conducted in healthy individuals concluded that no ε4 gene dose effects were present in the hippocampal volume or their change rates. Nonetheless, although we found significant effects when testing the additive model, we should again emphasize that our data were mainly driven by the *APOE*-ε4 group; therefore our data are partially in agreement with the previously cited studies. Our finding on the dose-dependent GMv reduction in the posterior hippocampus may represent one mechanism for the dose-dependent AD vulnerability exerted by *APOE*-ε4 [3,4].

The whole-brain analysis detected additional ε4 additive GMv reductions in three regions: the right caudate nucleus, the right cerebellar crus, and the right precentral gyrus.

Of these regions, the former two also displayed an interaction with age in a similar direction as for the posterior hippocampus, whereas the latter did not. Two previous studies reported caudate atrophy in healthy *APOE*-ε4 carriers [21,39]. In addition, the caudate and the crus of the cerebellum, together with other cortical areas, form part of the extended network subserving executive functions [40], and the volume of the cerebellar crus is specifically related to efficiency in multiple cognitive domains including executive functioning [41]. Of interest, this executive control network has been reported to be selectively affected in AD [42]. To test whether the *APOE*-ε4-allelic load would modulate the association between volumes in these regions and cognitive performance, we performed a post hoc analysis. We looked for the associations between GMv in these regions and executive function and sought for an interaction effect with *APOE*-ε4 allele load. We found significant interactions for the Digit-Symbol Substitution Test and Digit-Span Sequencing subscales of the Wechsler Adult Intelligence Scale in the caudate and cerebellar crus. In these areas, lower executive performance was associated to increased GMv in the ε4 homozygote group only, although no between-group differences were detected in executive function. On the other hand, no interactions were observed for episodic memory measurements. This effect may indicate that GMv changes in these areas could be related to the decline in executive function reported in preclinical AD stages [43]. However, to confirm this hypothesis, the impact of *APOE*-ε4 load on the neuroanatomical correlates of cognition calls for additional investigations and further studies shall address this on a brain connectivity network level, something which goes beyond the scope of the present article. Another possible interpretation would be that other effects of the *APOE*-ε4 allele that may precede or be independent of amyloid deposition impact executive function. In this respect, *APOE*-ε4 has been linked to impaired cholesterol delivery to the brain to sustain synaptic integrity and plasticity. Therefore, to discern whether the impact of *APOE*-ε4 load on the neuroanatomical correlates of cognition in healthy subjects is independent of the presence of neuropathological abnormalities, further studies with core AD biomarkers are needed. Our finding on the additive effect of *APOE*-ε4 in determining lower volume in the precentral gyrus is in line with another study reporting thinner sensorimotor cortex in children and adolescent carriers of the ε4 allele [44], although these regions are structurally affected only in the later stages of AD. The lack of interaction in our findings with age in the precentral gyrus suggests that this effect represents a genetically determined neuroanatomical trait rather than being associated to AD pathology. Nevertheless, the main effect in the study of Shaw et al. [44] was located in the entorhinal region, a brain region where we did not observe *APOE*-ε4-related alterations. A different longitudinal evolution with aging or the impact of early AD-related pathological events in this area could underlie such null finding.

Our hypothesis-driven analysis yielded a significant *APOE*-ε4 dose-dependent additive GMv increase in the right medial thalamus. This result was confirmed in the whole-brain analysis, which additionally revealed a dose-dependent GMv increase in the left middle occipital cortex and a genotypic (recessive with the ε4 allele) GMv increase in the right superior frontal cortex. The hypertrophy we detected in the superior frontal cluster is in line with one study reporting greater GMv in this area in healthy ε4-carriers compared to NC [21]. Our data are also consistent with two prior studies conducted in healthy subjects having a similar age range as our sample, which showed thicker frontal and occipital areas in *APOE*-ε4 carriers compared to NC [45,46]. Increased regional GMv in temporal areas has been previously described in amyloid-positive healthy individuals [47] who were hypothesized to reflect brain swelling associated to glial activation in preclinical AD stages [48]. However, given the lack of pathological and neuroinflammatory markers, we could not confirm this interpretation. Similarly, Johnson et al. [49] found greater GMv in the lateral parietal and right ventral temporal lobes in amyloid-positive cognitively healthy subjects. In individuals with intermediate amyloid levels, they also found increased fludeoxyglucose uptake in the thalamus. Additional multimodal longitudinal studies are needed to elucidate the temporal evolution of these neuroimaging correlates. The effect of *APOE*-ε4 on cerebral Aβ deposition in cognitively healthy individuals has been found to be dose-dependent and increased in homozygotes in extensive brain areas including the basal ganglia, as

well as frontal and occipital cortices [50]. In fact, at the mean age in our sample, about 50% of ε4 homozygotes are expected to harbor Aβ pathology while only 20% of the heterozygotes and <10% of the NC [51]. Therefore, our findings might represent an early event in the AD cascade in reaction to the presence of aggregated Aβ, in a process that eventually leads to GM atrophy, as already proposed earlier [30,49]. However, we did not observe a significant interaction with age in any of the brain regions where *APOE*-ε4 was associated to greater GMv, as would have been expected should these increments be associated with amyloid deposition. This could be explained by a lower effect size of inflammatory effects in brain structure as compared to neurodegeneration. Certainly, other possible *APOE*-related biological mechanisms other than Aβ aggregation could underlie our findings, such as defective neuronal pruning [52], initial compensatory response to neuronal stress, and progressive age-related loss of compensation [53,54] or broader processes related to accelerated aging [46]. Other explanations would include a selection bias in our sample. *APOE*-ε4 homozygotes are less likely to remain cognitively intact after the age of 60 years. Therefore, the inclusion criteria in our study could have favored the inclusion of older *APOE*-ε4 homozygotes with a higher cognitive or brain reserve, potentially driving our results.

On the other hand, we did not observe any effects in areas known to display reduced glucose metabolism in a dose-dependent manner with *APOE*-ε4 allele load, such as the precuneus, posterior cingulate, and parietotemporal areas, also known to be affected in AD [55]. This is in agreement with previous reports that studied the linkage between fludeoxyglucose positron emission tomography and GMvs in which the latter did not reach statistical significance independently [56].

When inspecting the contribution of five different haplotypes, our interaction data suggest that the group ε2/ε2 + ε2/ε3 displays relatively spared lifetime regional GMv values. Although our study is cross sectional, this suggests that these two haplotypes may be related to healthy aging and longevity. The assessment of this aspect goes beyond the scope of the present research and should be investigated in future prospective studies. When modeling the effects of each *APOE* haplotype on regional GMv, we found a pattern of results that confirmed the ε4 dose-dependent effects in all regions except the right superior frontal gyrus, as discussed previously. In addition, this analysis revealed that the greatest difference in regional GMv occurred between the ε2/ε2 + ε2/ε3 and ε4/ε4 genotype groups.

When considering the present study, one should be aware of the cross-sectional nature of our design, which makes difficult to interpret the results in terms of temporal evolution of the events because data were collected in a single time point. Although this represents a limitation of our study, it also encourages the development of more comprehensive longitudinal studies to be conducted in the healthy population, which shall address more specifically the relationships between *APOE*-ε4 load and aging in determining structural brain changes. Another limitation of our study is the lack of core AD (i.e., Aβ and tau) or other inflammatory biomarkers. Although our morphometrical data help to uncover the plausible mechanisms that relate *APOE* genotype and AD pathogenesis, we cannot clearly establish the biological processes underlying our findings in GMvs without specific biomarker data. Future studies shall take these parameters into consideration.

As a possible methodological development of our research, future studies shall consider the implementation of multivariate statistical approaches, which may reveal patterns of structural GM covariance and already proved insightful for the assessment of the age-related structural brain correlates [57].

Another important aspect is that our sampling strategy was designed to maximize the number of *APOE*-ε4 homozygotes. Therefore, the allele frequencies in our study are not representative of those of an unselected population, which prevents us from drawing any epidemiological conclusions.

In summary, we report region-specific additive and recessive main effects of the *APOE*-ε4 on GMvs in healthy individuals as well as interactions between *APOE* status and age in determining GMvs in these areas. Our data mirror previous findings of the ε4 additive effects on cortical degeneration in AD patients and suggest that the dose-dependent risk for AD conferred by *APOE*-ε4 may be reflected by altered GMvs already in healthy individuals.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2018.01.016.

RESEARCH IN CONTEXT

1. Systematic review: Apolipoprotein E (*APOE*)-ε4 confers dose-dependent increased risk for Alzheimer's disease (AD). In AD patients, *APOE*-ε4 exerts additive effects in determining the degree of hippocampal atrophy. However, only a few reports have assessed dose-dependent effects of the risk allele on brain morphology in healthy individuals at higher risk of AD.
2. Interpretation: We found additive effects of *APOE*-ε4 determining lower gray matter volume in the right hippocampus, caudate, precentral gyrus, and cerebellar crus. *APOE*-ε4 was also associated to greater gray matter volume in the right thalamus, left occipital gyrus, and right frontal cortex. Our data suggest that the dose-dependent vulnerability induced by *APOE*-ε4 may be reflected on the brain morphological level in regions that are critical for AD pathophysiology.
3. Future directions: Future studies shall take into account AD biomarkers such as Aβ or tau protein to better characterize the underlying biological mechanisms through which *APOE*-ε4-allelic load predisposes individuals to AD.

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